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# Dynamic analysis of a genomic island in *Magnetospirillum* sp. strain AMB-1 reveals how magnetosome synthesis developed

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**Abstract The entire structure of a 98 kb genomic region that abounds in genes related to magnetosome synthesis was first described in the *Magnetospirillum* sp. strain AMB-1. The deletion of this 98 kb genomic region and the circular form after excision from the chromosome was detected by PCR amplification. This strongly suggests that the region has undergone a lateral gene transfer. The region has the characteristics of a genomic island: low GC content, location between two repetitive sequences, and the presence of an integrase in the flanking region of the first repetitive sequence. This 98 kb genomic region has the potential for transfer by the integrase activity. Comparative genome analysis revealed other regions with a high concentration of orthologs in magnetic bacteria besides the 98 kb region, and magnetosome synthesis seemed to need not only the exogenous 98 kb region, but also other orthologs and individually originating genes.**


*Keywords:* Magnetic bacteria; Magnetosome synthesis; Genomic island; Comparative analysis; *Magnetospirillum* sp. strain AMB-1

## 1. Introduction

Genome sequences from a large number of organisms have been elucidated, and a variety of life phenomena have been interpreted using information from these genome sequences. Recently, genomic regions with aggregating genes related to the same life phenomenon have been found. These regions are called genomic islands (GEIs), and represent what have been previously been transferred by other mobile genetic elements and are present in certain bacteria but are absent in most closely related variants [1]. GEIs carry one or more genes that can increase the adaptability and versatility of the bacterium, are frequently associated with tRNA genes, and are flanked by repeat structures. They contain mobility genes coding for integrase or transposons that are required for chromosomal integration and excision. GEIs contribute to the dynamic character of bacterial chromosomes and can be excised from the chromosome and transferred to other recipients. Such exogenous gene transfer is closely associated with the acquisition or development of environmental adaptation capability. Various GEIs were identified in the iron uptake system in *Bacillus cereus* [2], the symbiosis island in *Methorhizobium loti* [3], and the resistance to multiple antibiotics in *Shigella flexneri* [4]. A GEI relating to magnetosome production in magnetic bacteria was suggested in *Magnetospirillum gryphiswaldense* MSR-1. It was called the ''magnetosome island'' [5]. Past acquisition of such a genomic island could contribute to magnetosome synthesis, and lead to a magneto-aerotaxis that is directional along geomagnetic field lines instead of a normal aerotaxis that is randomly seeking the optimal oxygen concentration in an oxygen gradient. It could be hypothesized that the increased efficiency of movement by such a magneto-aerotaxis [6] resulted in an evolutionary advantage.

Magnetic bacteria contribute to the global iron cycle by acquiring iron and converting it into magnetite ($Fe_3O_4$) or greigite ($Fe_3S_4$), which accumulates in intracellular structures known as magnetosomes [7,8]. Recently, the genome sequence of the magnetic bacterium *Magnetospirillum* sp. strain AMB-1 was completed [9]. The genome of *Magnetospirillum* sp. strain AMB-1 consists of a single circular chromosome of 4967148 bp and 4559 predicted open reading frames (ORFs), and includes many vestiges of past exogenous gene transfer, such as insertion sequence (IS) elements, integrases, and large regions containing phage-coding genes. In whole-genome sequence analysis, a 98 kb deletion from the chromosome has been observed. The region was low GC content, included genes encoding magnetosome-specific proteins, and was flanked by 1.1 kb repetitive sequence [9]. It seemed possible that the corresponding region included many homologues between three magnetic bacteria, *Magnetospirillum magnetotacticum* MS-1, *Magnetococcus* MC-1, and *M. gryphiswaldense* MSR-1 [10]. The region has been identified as a ''magnetosome island'' which is deleted in non-magnetic spontaneous mutants of *M. gryphiswaldense* MSR-1 and has been partially sequenced [5]. However, the complete structure of the region and the deletion mechanism remains unknown.

In this manuscript, the deletion in non-magnetic spontaneous mutants of *Magnetospirillum* sp. strain AMB-1 was detected by PCR amplification using a primer set designed from the whole genome sequence. Furthermore, the deletion region was confirmed as 98 kb of circular form suggesting a lateral transfer occurred in the past. Results from comparative genome analysis, i.e., the distribution of orthologs in magnetic bacteria, showed the mechanism of acquisition of magnetosome synthesis.

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*Abbreviations:* GEIs, genomic islands

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## 2. Materials and methods

### 2.1. Bacterial strain and culture conditions

*Magnetospirillum* sp. strain AMB-1 (ATCC700264) was used in this study. Wild-type and non-magnetic spontaneous mutant were grown anaerobically at 25 °C in modified magnetic spirillum growth medium (MSGM) at pH 6.75 until stationary phase [11]. Solid medium contained 1% agar.

### 2.2. Preparation and growth check of non-magnetic spontaneous mutants

In order to obtain non-magnetic spontaneous mutant reproducibly, *Magnetospirillum* sp. strain AMB-1 cells that synthesized magnetosomes in liquid culture were harvested with a neodymium–iron–boron magnet closely attached to a 50 ml-culture flask. The remaining cells, those not responding to the magnet, were diluted to $10^4$ cells/ml and plated on MSGM agar medium. After 3 weeks, the non-magnetic cells formed white colonies and were observed by transmission electron microscope (TEM) as described previously [12].

### 2.3. PCR amplification and sequencing for determination of deletion

The deletion of the 98 kb region from the genome and the closed circular form of the region were detected by PCR amplification. The strategy of PCR amplification is indicated in Fig. 1a. PCR amplification using LA *Taq* polymerase with GC buffer II (Takara, Shiga, Japan) and each primer set listed in Table 1 was performed. Direct PCR was employed using $10^3$ cells of magnetic harvested wild-type and non-magnetic spontaneous mutants of *Magnetospirillum* sp. AMB-1 for deletion detection. For the circular form detection, 1–10 ng of extracted genomic DNA from a stationary-phase culture was used as the PCR template. Genomic DNA from *Magnetospirillum* sp. AMB-1 wild-type and non-magnetic spontaneous mutants was extracted according to a standard protocol [13]. A 1.2 kb PCR fragment was cloned into the vector pGEM-T-easy (pGEM-T-easy Vector System, PROMEGA, WI, USA) and sequenced using an automatic DNA sequencer ABI 3100 (Perkin–Elmer Co., CA, USA).

### 2.4. Comparative genome analysis

All completed and draft genome sequences of magnetic or non-magnetic bacteria listed in Supplementary Table 1 were annotated in the same manner as *Magnetospirillum* sp. strain AMB-1 [9]. A total of 4559 deduced genes from the whole genome of *Magnetospirillum* sp. strain AMB-1 were compared with magnetic or non-magnetic bacteria at the amino acid level using the program BLASTP and the ortholog clustering method [14]. The threshold of 1e − 10 was applied for all bacteria.

### 2.5. Nucleotide sequence accession numbers

The sequence of the complete genome of *Magnetospirillum* sp. AMB-1 is available under DDBJ Accession number AP007255.

## 3. Results

### 3.1. Isolation of non-magnetic spontaneous mutants

*Magnetospirillum* sp. strain AMB-1 can grow on a solid plate and forms colonies that are black due to intracellular magnetite, while non-magnetic spontaneous mutants form white colonies. One white colony was generated to 50 black colonies after liquid cultivation. Non-magnetic mutants were isolated in order to confirm reproducibility. Three weeks after

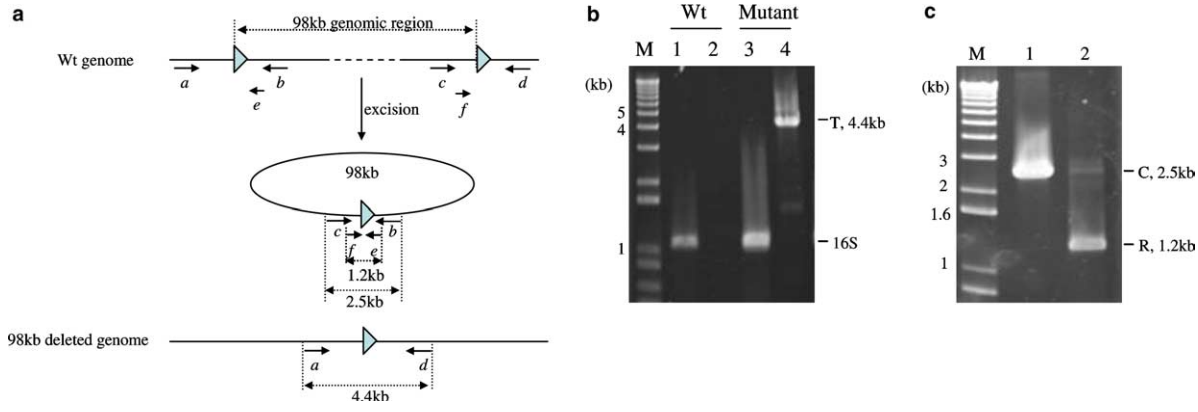


Fig. 1. Analysis of the 98 kb deleted region in *Magnetospirillum* sp. strain AMB-1. (**a**) Schematic depiction of deletion. DNA element of 98 kb was deleted from the genome by integrase activity, and became a circular form. The circular form and 98 kb genomic region were detected as fragments by PCR and agarose gel electrophoresis. Triangles represent repetitive sequences, arrows represent primer positions designed in this study. (**b**) Agarose gel electrophoresis of PCR products of AMB-1 wild-type (lanes 1 and 2) and mutant (lanes 3 and 4). 16S rDNA specific primers (positive control, lanes 1 and 3) and primers depicted in *a* and *d* (lanes 2 and 4). (**c**) Agarose gel electrophoresis of PCR products obtained using primers depicted in *b* and *c* (lane 1), and results from PCR of the purified product of lane 1 using primers complimentary to repetitive sequences (lane 2). Approximate sizes (kb) of the fragments are shown the left of the photo. The explanation of these fragments are shown the right. *T*, trace region of 98 kb deletion. 16S, 16S rDNA. C, 2.5 kb fragment corresponding to the closed site of 98 kb deleted region after excision from the chromosome. R, a repetitive sequence on the inside of the 2.5 kb fragment.

Table 1
PCR primers used to detect 98 kb deletion and the circular form

| Objective for detection | PCR primer sequence (5′ to 3′) | | PCR annealing temperature (°C) | Product size |
|---|---|---|---|---|
| | Forward | Reverse | | |
| A part of 16S rDNA in AMB-1 | ACACGTGGGAATATACCTCTTGGTGGGG | CACCATTGTAGCACGTGTGTAGCCCAGC | 65 | 1032 |
| Deletion of 98 kb region | CGCCGTCCATTCTCCAACTGATC (a) | ATGGCATATCGAGCGCCACCTTCT (d) | 65 | 4434 |
| Circular form of 98 kb region | TGTCCAGAACGTCCATTCCATGAGC (b) | ATTGGTCATGGACGAAGTCCAGC (c) | 52 | 2509 |
| 1.1 kb repetitive sequence | AGGTTCGCTATCCACCGTGATCATC (e) | AACAGCCGACGATAGCCGAAA (f) | 54 | 1211 |

The letter in parentheses is corresponding to the primer name of Fig. 1.

plating of non-magnetic cells, about 80 colonies were formed on a plate, all of them were white or light auburn. Results from observation by TEM, revealed that white colonies had no magnetosomes. Several white colonies were randomly selected and cultivated in MSGM medium to use in subsequent experiments. Isolated mutants showed increased growth compared to wild-type cells. The growth reached a plateau at 15 h faster than wild-type, and the number of the cells at plateau was about 1.2 times that of wild-type.

### 3.2. Detection of a deletion of the 98 kb region

Genome analysis of non-magnetic mutants of *Magnetospirillum* sp. strain AMB-1 revealed a large deletion of the 98 kb genomic region. The *XerC* gene homolog (*amb0926*) is located 2 and 102 kb upstream of two identical 1133-bp sequences. Both 1133-bp repetitive sequences include truncated IS elements. It has previously been speculated that *amb0926* and the 1133 bp repetitive sequences may be associated with the deletion of the 98 kb genomic region [9], similar to the observation by Sakellaris et al. [15].

In order to detect the deletion, primer sets were designed on both sides flanking the approximate region where the deletion might occur. In the case of the occurrence of a deletion, a 4.4 kb fragment is amplified as the distance between the two primers becomes shortened. Conversely, if no deletion occurs, there will be no PCR amplification because the primer set is separated by a distance of 98 kb in wild-type genome. Results from PCR analysis showed the 4.4 kb fragment, which means non-magnetic spontaneous mutants lost the 98 kb genomic region (Fig. 1b). Further sequence analysis showed the deletion occurred at position nt 997 403–1 095 894 in the wild-type. This deletion occurred in all cells of 20 white colonies isolated as non-magnetic cell, in this study.

Integrase-mediated excision usually leads to circular episomal intermediates that are substrates for conjugal transfer or packaging into phage particles [15]. Circular forms indicate that the transposable region has been derived by lateral transfer in the past. The free circular form excised from the genome was investigated by PCR amplification of the hypothetical junction between the left and right boundaries of the deleted region (Fig. 1c). The results showed a 2.5 kb band corresponding to the size of a region including the hypothetical junction. Furthermore, a 2.5 kb fragment of PCR product was isolated by gel extraction and utilized as a template for PCR to detect repetitive sequences. As a result, 1.2 kb-band was amplified and subsequently cloned. The sequence of this fragment corresponded to the hypothetical junction exactly indicating that it had been a closed circular form of the genomic region after excision from the chromosome.

These structural and dynamics results suggest that the 98 kb region is a kind of genomic island related to magnetosome synthesis, and that it has been derived by lateral transfer. The free closed island was not duplicated in the cell because there are no structures like duplication-ori. Therefore, during cell division, one of the resulting cells has no island. Non-magnetic spontaneous mutants are thought to be generated in this manner.

### 3.3. Structure of the genomic region

The genome structure of the 98 kb deleted region is represented in Fig. 2. It is flanked by 1.1 kb repetitive sequences

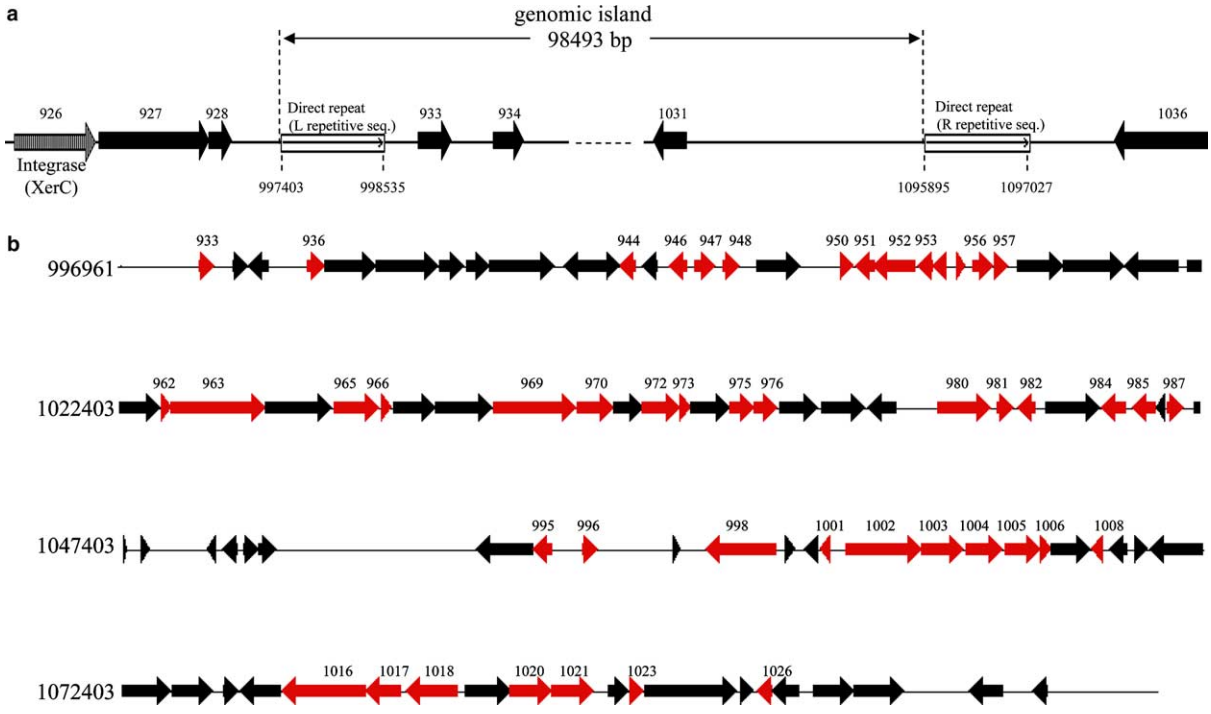


Fig. 2. Genome structure of the 98 kb genomic island in *Magnetospirillum* sp. strain AMB-1. (a) Schematic view of the genome structure among nt 994 000–1 099 000 in the AMB-1 genome. Arrows on the line represent predicted ORFs. Numbers above the arrows represent each gene ID (i.e., amb0926, amb1035). The 1.1 kb repetitive sequence is depicted as a square. (b) Deduced gene organization in the 98 kb genomic island. The meanings of arrows and numbers are similar to Fig. 2a. Red arrows represent common genes in magnetic bacteria.

Table 2
Feature of deduced genes in the translocatable 98 kb genomic island in *Magnetospirillum* sp. starin AMB-1

| Gene ID | Predicted function[a] | Best hit organisms in non-magnetic bacteria[b] | *E* value[c] | Homologues in magnetic bacteria[b] | *E* value | Paralogues in AMB-1 | *E* value |
|---|---|---|---|---|---|---|---|
| amb0933[d] | Hypothetical protein | – | | – | 2e − 62 | – | – |
| amb0934[e] | Hypothetical protein | – | | – | – | – | – |
| amb0935 | Hemerythrin-like protein CAC0069[f] | Clostridium tetani E88 (AE015944-70) | 1e − 12 | MSR-1(ORF13)<br>MS-1 (ZP_00053162.2)<br>MC-1 (ZP_00290955.1) | 1e − 45<br>5e − 26<br>5e − 13 | amb0985 | 1e − 26 |
| amb0936[d] | Hypothetical protein | – | | MS-1<br>MS-1 (ZP_00053411.2) | 4e − 74<br>8e − 69 | – | – |
| amb0937 | High-affinity $Fe^{2+}$/$Pb^{2+}$ permease[f] | *Yersinia pestis* (AL031866-38) | 1e − 68 | MS-1 (ZP_00050420.1) | 4e − 44 | amb1681 | 1e − 14 |
| amb0938 | Polyferredoxin | *Chromobacterium violaceum* (AE016912-229) | 4e − 62 | MV-1(AY587957-1)<br>MS-1 (ZP_00055323.2) | e − 147<br>8e − 61 | amb4413 | 7e − 61 |
| amb0939 | Uncharacterized protein probably involved in high-affinity $Fe^{2+}$ transport[f] | *Bordetella pertussis* (BX640414-150) | 1e − 42 | MV-1(AY588947-1)<br>MS-1 (ZP_00054566.1) | 1e − 44 | amb0940 | 1e − 57 |
| amb0940 | Uncharacterized protein probably involved in high-affinity $Fe^{2+}$ transport[f] | *Burkholderia mallei* (CP000011-716) | 2e − 39 | MV-1(AY588947-1)<br>MS-1 (ZP_00054566.1) | 6e − 34<br>6e − 26 | amb0939 | 1e − 57 |
| amb0941 | Carbohydrate-selective porin | *Burkholderia pseudomallei* (BX571965-300) | 6e − 85 | MS-1 (ZP_00053838.2) | 3e − 98 | amb3444 | 2e − 97 |
| amb0942 | Hypothetical protein | – | | – | – | – | – |
| amb0943 | Membrane-fusion protein | *Erwinia carotovora* subsp. (BX950851-793) | 2e − 47 | MS-1 (ZP_00053376.2)<br>MC-1 (ZP_00291059.1) | 5e − 75<br>2e − 63 | amb1143 | 2e − 74 |
| amb0944[d] | Hypothetical protein | – | | MS-1(original annotation) | 5e − 58 | – | – |
| amb0945[e] | Hypothetical protein | – | | – | – | – | – |
| amb0946[d] | Hypothetical protein | *Gloeobacter violaceus* (AP006579-67) | 6e − 05 | MS-1 (ZP_00053412.2) | 3e − 70 | – | – |
| amb0947[d] | Hypothetical protein | – | | MS-1 (ZP_00208479.1) | 6e − 85 | – | – |
| amb0948[d] | FOG: CheY-like receiver[g] | *Yersinia enterocolitica* (AF354753-4) | 4e − 16 | MS-1 (ZP_00208480.1)<br>MC-1 (ZP_00289896.1) | 2e − 57<br>2e − 16 | amb1370 | 7e − 16 |
| amb0949 | Transposase and inactivated derivatives | *Caulobacter crescentus* CB15 (AE005940-4) | 2e − 95 | MS-1 (ZP_00052814.2) | 6e − 91 | amb1034, amb0931 | 9e − 17 |
| amb0950[d] | Hypothetical protein | – | | MS-1 (ZP_00208481.1) | 2e − 45 | – | – |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| amb0951[d] | Bacterial magnetic particle specific iron-binding protein(*mms13*)[f] | – | | MSR-1(ORF8, *mamC*)[h]<br>MS-1 (ZP_00053414.2)<br>MC-1 (ZP_00288328.1) | 3e − 53<br>4e − 64<br>8e − 32 | – | – |
| amb0952[d] | Bacterial magnetic particle specific iron-binding protein(*mms7*)[f] | *Galleria mellonella* (AF095239-1) | 7e − 10 | MSR-1(ORF7, *mamD*)[h]<br>MS-1 (ZP_00053415.1)<br>MC-1 (ZP_00289784.1) | e − 169<br>e − 170<br>6e − 31 | amb0400 | 6e − 52 |
| amb0953[d] | Hypothetical protein | – | | MSR-1(ORF6, *mamF*)[h]<br>MS-1 (ZP_00053416.1)<br>MC-1 (ZP_00288335.1) | 2e − 48<br>2e − 61<br>7e − 22 | amb0957, amb1026, amb412 | 3e − 38 |
| amb0954[d] | Bacterial magnetic particle specific iron-binding protein homologue[f] | *Pinctada fucata* (D86074-1) | 1e − 05 | MSR-1(ORF5, *mamG*)[h]<br>MS-1 (ZP_00053417.2) | 4e − 22<br>1e − 51 | amb1027, amb0952, amb0956 | 2e − 19 |
| amb0955[d] | Hypothetical protein | – | | – | – | – | – |
| amb0956[d] | Bacterial magnetic particle specific iron-binding protein(*mms6*)[f] | *Oryza sativa* (AP005311-18) | 2e − 09 | MSR-1(ORF4)[h]<br>MS-1 (ZP_00053419.2)<br>MC-1 (ZP_00287884.1) | 2e − 67<br>9e − 68<br>4e − 16 | amb1027, amb400, amb0954, | 4e − 20 |
| amb0957[d] | Hypothetical protein | – | | MSR-1(ORF3)[h]<br>MS-1 (ZP_00053420.2)<br>MC-1 (ZP_00288335.1) | 1e − 53<br>1e − 48<br>7e − 24 | amb0953, amb0412, amb1026 | 2e − 38 |
| amb0958[d] | Uncharacterized protein conserved in bacteria | *Sinorhizobium meliloti* (AL591792-256) | 3e − 09 | MSR-1(ORF2)[h]<br>MS-1 (ZP_00053421.2) | e − 133<br>e − 147 | amb4422 | 2e − 22 |
| amb0959 | Uncharacterized membrane-bound protein | *Agrobacterium tumefaciens* (AE008179-2) | 6e − 31 | MSR-1(ORF1)[h]<br>MS-1 (ZP_00208607.1) | 3e − 85<br>0.0 | amb4423 | 5e − 41 |
| amb0960 | Predicted membrane protein | *Streptomyces avermitilis* (AP005041-237) | 2e − 61 | MSR-1(ORF16)[h]<br>MS-1 (ZP_00053281.2) | 0.0<br>0.0 | – | – |
| amb0961 | Permeases of the major facilitator superfamily | *Chlorobium tepidum* TLS (AE012904-9) | 6e − 40 | MSR-1(ORF17, *mamH*)[h]<br>MS-1 (ZP_00053280.2)<br>MC-1 (ZP_00288324.1) | 0.0<br>0.0<br>e − 125 | amb1016 | 9e − 95 |
| amb0962[d] | Hypothetical protein | – | | MSR-1(ORF18, *mamI*)[h]<br>MS-1 (ZP_00208239.1)<br>MC-1 (ZP_00288325.1) | 2e − 25<br>1e − 34<br>3e − 17 | – | – |
| amb0963[d] | Trypsin-like serine proteases, typically periplasmic, contain C-terminal PDZ domain | *Mesorhizobium loti* (AP003005-185) | 2e − 42 | MSR-1(ORF19, *mamE*)[h]<br>MS-1 (ZP_00054403.1)<br>MC-1 (ZP_00288326.1) | 0.0<br>0.0<br>3e − 91 | amb1002 | 0.0 |
| amb0964 | Periplasmic protein TonB, links inner and outer membranes | *Streptomyces avermitilis* (AP005038-252) | 2e − 35 | MSR-1(ORF20, *mamJ*)[h]<br>MS-1 (ZP_00054404.2)<br>MC-1 (ZP_00289916.1) | 7e − 86<br>e − 125<br>5e − 19 | amb1003 | 5e − 18 |
| amb0965[d] | Actin-like ATPase involved in cell morphogenesis | *Methanopyrus kandleri* AV19 (AE010316-8) | 1e − 20 | MSR-1(ORF21, *mamK*)[h]<br>MS-1 (ZP_00054405.2)<br>MC-1 (ZP_00288334.1) | 0.0<br>0.0<br>e − 101 | amb3513 | 2e − 13 |

(*continued on next page*)

Table 2 (*continued*)

| Gene ID | Predicted function[a] | Best hit organisms in non-magnetic bacteria[b] | *E* value[c] | Homologues in magnetic bacteria[b] | *E* value | Paralogues in AMB-1 | *E* value |
|---|---|---|---|---|---|---|---|
| amb0966[d] | Hypothetical protein | – | | MSR-1(ORF22, *mamL*)[h]<br>MC-1 (ZP_00208240.1) | 4e − 30<br>1e − 32 | amb0407 | 5e − 25 |
| amb0967 | Predicted Co/Zn/Cd cation transporters | *Bacillus halodurans* C-125 (AP001509-150) | 8e − 33 | MSR-1(ORF23, *mamM*)[h]<br>MS-1 (ZP_00054406.2)<br>MC-1 (ZP_00288337.1) | e − 171<br>e − 168<br>3e − 77 | amb1007, amb0974 | 8e − 31 |
| amb0968 | $Na^+/H^+$ antiporter NhaD and related arsenite permeases | *Bacillus anthracis* str. Ames (AE017039-310) | 5e − 44 | MSR-1(ORF24, *mamN*)[h]<br>MS-1 (ZP_00054407.1) | 0.0<br>0.0 | amb0742 | 3e − 20 |
| amb0969[d] | Trypsin-like serine proteases, typically periplasmic, contain C-terminal PDZ domain | *Caulobacter crescentus* CB15 (AE005803-4) | 5e − 17 | MSR-1(ORF25, *mamO*)[h]<br>MS-1 (ZP_00054408.1)<br>MC-1 (ZP_00288338.1) | 0.0<br>0.0<br>e − 101 | amb1004 | e − 155 |
| amb0970[d] | Hypothetical protein | – | | MSR-1(ORF26, *mamP*)[h]<br>MS-1 (ZP_00054409.1)<br>MC-1 (ZP_00288339.1) | e − 120<br>e − 157<br>1e − 40 | – | – |
| amb0971 | FOG: TPR repeat *(mms24)* Essential for magnetosome formation | *Methanosarcina mazei* Go1 (AE013478-3) | 2e − 14 | MSR-1(ORF27, *mamA*)[h]<br>MS-1 (ZP_00054410.1)<br>MC-1 (ZP_00288340.1) | e − 118<br>e − 120<br>9e − 37 | amb2357 | 2e − 12 |
| amb0972[d] | Uncharacterized conserved protein | *Thermotoga maritima* MSB8 (AE001759-4) | 1e − 18 | MSR-1(ORF28, *mamQ*)[h]<br>MS-1 (ZP_00053530.2)<br>MC-1 (ZP_00289800.1) | e − 125<br>e − 138<br>5e − 15 | amb1005 | e − 154 |
| amb0973[d] | Hypothetical protein | – | | MSR-1(ORF29, *mamR*)[h]<br>MS-1 (ZP_00054412.1) | 2e − 35<br>3e − 42 | amb1006 | 9e − 43 |
| amb0974 | Predicted Co/Zn/Cd cation transporters | *Bacillus subtilis* subsp. *subtilis* (Z99106-181) | 2e − 38 | MSR-1(ORF30, *mamB*)[h]<br>MS-1 (ZP_00054413.1)<br>MC-1 (ZP_00289797.1) | e − 157<br>e − 166<br>2e − 79 | amb1007 | e − 166 |
| amb0975[d] | Hypothetical protein | – | | MSR-1(ORF31, *mamS*)[h]<br>MS-1 (ZP_00054414.2)<br>MC-1 (ZP_00289796.1) | 1e − 73<br>7e − 92<br>2e − 16 | amb1017 | 2e − 11 |
| amb0976[d] | Hypothetical protein | – | | MSR-1(ORF32, *mamT*)[h]<br>MS-1 (ZP_00054415.1)<br>MC-1 (ZP_00289795.1) | 5e − 85<br>9e − 94<br>1e − 27 | – | – |
| amb0977 | Sphingosine kinase and enzymes related to eukaryotic diacylglycerol kinase | *Mesorhizobium loti* (AP003017-148) | 8e − 26 | MSR-1(ORF33, *mam U*)[h]<br>MS-1 (ZP_00054416.1) | e − 125<br>e − 166 | amb2502 | 2e − 38 |
| amb0978 | Predicted Co/Zn/Cd cation transporters | *Methanosarcina mazei* Go1 (AE013526-7) | 2e − 31 | MSR-1(ORF30, *mamB*)[h]<br>MS-1 (ZP_00054417.2)<br>MC-1 (ZP_00289797.1) | 7e − 53<br>e − 164<br>2e − 51 | amb1007, amb0974 | 3e − 56 |
| amb0979 | Hypothetical protein | – | | MS-1 (ZP_00054419.1) | 2e − 66 | – | – |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| amb0980[d] | FOG: CheY-like receiver[g] | *Caulobacter crescentus* CB15 (AE005979-9) | 5e − 11 | MSR-1(ORF12)[h]<br>MS-1 (ZP_00054420.2) | 3e − 17<br>0.0 | amb3331 | 5e − 21 |
| amb0981[d] | Hypothetical protein | – | | MS-1 (ZP_00054421.1) | 1e − 60 | – | – |
| amb0982[d] | Predicted transcriptional regulators | *Sinorhizobium meliloti* (AL591785-153) | 2e − 06 | MS-1 (ZP_00054422.1) | 3e − 62 | – | – |
| amb0983 | Hypothetical protein | *Bradyrhizobium japonicum* (AP005955-60) | 2e − 06 | MSR-1(ORF12)[h]<br>MS-1 (ZP_00053164.2) | 3e − 10<br>e − 105 | amb3331 | 6e − 72 |
| amb0984[d] | Hypothetical protein | – | | MS-1 (ZP_00053163.1) | 2e − 95 | – | – |
| amb0985[d] | Hemerythrin-like protein PA1673.[f] | *Clostridium tetani* E88 (AE015944-70) | 2e − 10 | MSR-1(ORF13)[h]<br>MS-1 (ZP_00053162.2) | 9e − 24<br>2e − 83 | amb0935 | 1e − 26 |
| amb0986[e] | Hypothetical protein | – | | – | – | – | – |
| amb0987[d] | Hypothetical protein | – | | – | – | – | – |
| amb0988[e] | Hypothetical protein | – | | – | – | amb0922, amb2077, amb2184 | 8e − 38 |
| amb0989[e] | Hypothetical protein | – | | – | – | – | – |
| amb0990[e] | Hypothetical protein | – | | – | – | – | – |
| amb0991[e] | Hypothetical protein | – | | – | – | amb2187 | 3e − 17 |
| amb0992[e] | Bacterial nucleoid DNA-binding protein | *Methylococcus capsulatus* (AE017282-510) | 5e − 25 | MS-1 (ZP_00208527.1)<br>MC-1 (ZP_00288474.1) | 1e − 42<br>6e − 23 | amb0923, amb2181, 1mb2079, | 6e − 46 |
| amb0993 | Transcriptional regulatory protein ros | *Mesorhizobium loti* (AP003015-254) | 1e − 32 | MS-1 (ZP_00055186.1)<br>MC-1 (ZP_00288070.1) | 1e − 35<br>1e − 20 | amb2080, amb0924 | 6e − 63 |
| amb0994 | Methyl-accepting chemotaxis protein[g] | *Rhodopseudomonas palustris* (BX572606-300) | 2e − 78 | MS-1 (ZP_00055894.1)<br>MC-1 (ZP_00288976.1) | e − 100<br>2e − 44 | amb2196, amb3701, amb4008, | 0.0 |
| amb0995[d] | FOG: PAS/PAC domain[g] | *Rhodopseudomonas palustris* (BX572606-192) | 7e − 43 | MS-1 (ZP_00208884.1) | 3e − 79 | amb2197 | 5e − 82 |
| amb0996[d] | FOG: CheY-like receiver[g] | *Thermosynechococcus elongatus* (AP005370-259) | 8e − 08 | MS-1 (ZP_00055002.1) | 5e − 45 | amb1215 | 4e − 45 |
| amb0997 | Hypothetical protein | – | | – | – | amb2195 | 9e − 21 |
| amb0998[d] | Uncharacterized low-complexity proteins | *Gloeobacter violaceus* (AP006578-72) | 4e − 30 | MS-1 (ZP_00053526.2)<br>MC-1 (ZP_00290177.1) | 0.0<br>3e − 19 | amb3384 | 1e − 17 |
| amb0999[e] | Hypothetical protein | – | | – | – | – | – |
| amb1000[e] | Hypothetical protein | – | | – | – | – | – |
| amb1001[d] | Hypothetical protein | – | | MS-1(original annotation) | 9e − 35 | – | – |

(*continued on next page*)

Table 2 (*continued*)

| Gene ID | Predicted function[a] | Best hit organisms in non-magnetic bacteria[b] | *E* value[c] | Homologues in magnetic bacteria[b] | *E* value | Paralogues in AMB-1 | *E* value |
|---|---|---|---|---|---|---|---|
| amb1002[d] | Trypsin-like serine proteases, typically periplasmic, contain C-terminal PDZ domain | *Mesorhizobium loti* (AP003005-185) | 3e − 46 | MSR-1(ORF19, *mamE*)[h]<br>MS-1 (ZP_00053527.1)<br>MC-1 (ZP_00288326.1) | 0.0<br>0.0<br>6e − 81 | amb0963, amb0410, amb3481, | 0.0 |
| amb1003[d] | FraH protein. | *Ralstonia solanacearum* (AL646081-82) | 2e − 14 | MSR-1(ORF20, *mamJ*)[h]<br>MS-1 (ZP_00053528.2) | 1e − 15<br>e − 168 | amb0964 | 3e − 18 |
| amb1004[d] | Trypsin-like serine proteases, typically periplasmic, contain C-terminal PDZ domain | *Caulobacter crescentus* CB15 (AE005803-4) | 1e − 17 | MSR-1(ORF25, *mamO*)[h]<br>MS-1 (ZP_00053529.2)<br>MC-1 (ZP_00288338.1) | e − 141<br>e − 162<br>7e − 30 | amb0969 | e − 155 |
| amb1005[d] | Uncharacterized conserved protein | *Thermotoga maritima* MSB8 (AE001759-4) | 8e − 19 | MSR-1(ORF28, *mamQ*)[h]<br>MS-1 (ZP_00053530.2)<br>MC-1 (ZP_00289800.1) | e − 125<br>e − 138<br>5e − 15 | amb0972 | e − 154 |
| amb1006[d] | Hypothetical protein | – | | MSR-1(ORF29, *mamR*)[h]<br>MS-1 (ZP_00054412.1) | 2e − 35<br>3e − 42 | amb0973 | 9e − 43 |
| amb1007 | Predicted Co/Zn/Cd cation transporters | Bacillus subtilis subsp. subtilis (Z99106-181) | 2e − 38 | MSR-1(ORF30, *mamB*)[h]<br>MS-1 (ZP_00054413.1)<br>MC-1 (ZP_00289797.1) | e − 157<br>e − 166<br>2e − 79 | amb0974 | e − 166 |
| amb1008[d] | Hypothetical protein | – | | MS-1 (ZP_00052635.2) | 5e − 38 | – | – |
| amb1009 | $Fe^{2+}/Zn^{2+}$ uptake regulation proteins[f] | *Caulobacter crescentus* CB15 (AE005680-1) | 3e − 49 | MS-1 (ZP_00052636.2) | 2e − 69 | amb4460 | 5e − 63 |
| amb1010[e] | Hypothetical protein | – | | – | – | – | – |
| amb1011[e] | Hypothetical protein | – | | – | – | – | – |
| amb1012 | Cation transport ATPase | *Pseudomonas putida* (AF333961-2) | e − 145 | MS-1 (ZP_00053714.2)<br>MC-1 (ZP_00289962.1) | 0.0<br>7e − 86 | amb1807 | e − 100 |
| amb1013 | Co/Zn/Cd efflux system component | *Sinorhizobium meliloti* (AL591789-68) | 9e − 95 | MS-1 (ZP_00054724.1)<br>MC-1 (ZP_00290474.1) | 0.0<br>6e − 81 | amb1234 | e − 137 |
| amb1014 | Uncharacterized conserved protein | *Gloeobacter violaceus* PCC 7421 (AP006582-176) | 9e − 37 | MS-1 (ZP_00054723.1) | 2e − 62 | amb3269 | 2e − 52 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| amb1015 | Cell division GTPase | *Anaplasma phagocytophilum* (AF221945-1) | e – 127 | MS-1 (ZP_00054722.2)<br>MC-1 (ZP_00290632.1) | e – 171<br>2e – 98 | amb3854 | e – 142 |
| amb1016[d] | Permeases of the major facilitator superfamily | *Chlorobium tepidum* TLS (AE012904-9) | 1e – 34 | MSR-1(ORF17, *mamH*)[h]<br>MS-1 (ZP_00208169.1)<br>MC-1 (ZP_00289786.1) | 5e – 92<br>0.0<br>0.0 | amb0961 | 1e – 94 |
| amb1017[d] | Hypothetical protein | – | | MSR-1(ORF19, *mamE*)[h]<br>MS-1 (ZP_00208168.1)<br>MC-1 (ZP_00289785.1) | 3e – 10<br>e – 152<br>3e – 44 | amb0410 | 5e – 13 |
| amb1018[d] | Methyl-accepting chemotaxis protein[g] | *Vibrio cholerae* O1 biovar eltor (AE004425-5) | 8e – 06 | MS-1 (ZP_00054719.2) | 0.0 | – | – |
| amb1019 | Hypothetical protein | *Thermoplasma acidophilum* (AL445064-164) | 2e – 40 | MS-1 (ZP_00054718.2) | 0.0 | amb1028 | e – 115 |
| amb1020[d] | Hypothetical protein | – | | MS-1 (ZP_00054717.1) | e – 179 | – | – |
| amb1021[d] | Serine/threonine protein kinase | *Burkholderia pseudomallei* (BX571965-578) | 2e – 28 | MS-1 (ZP_00054716.1) | 0.0 | amb1029 | e – 103 |
| amb1022 | Hypothetical protein | *Bradyrhizobium japonicum* (AP005964-112) | 2e – 11 | MS-1 (ZP_00208167.1) | 4e – 40 | amb3444 | 2e – 24 |
| amb1023[d] | $Fe^{2+}$ transport system protein FeoA[f] | *Lactococcus lactis* subsp. *lactis* (AE006256-8) | 7e – 08 | MS-1 (ZP_00208166.1) | 3e – 54 | – | – |
| amb1024 | $Fe^{2+}$ transport system protein FeoB[f] | *Porphyromonas gingivalis* W83 (AE017176-133) | e – 151 | MS-1 (ZP_00054713.1)<br>MC-1 (ZP_00287878.1) | 0.0<br>e – 132 | amb2731 | 1e – 99 |
| amb1025[e] | Hypothetical protein | – | | – | – | – | – |
| amb1026[d] | Hypothetical protein | – | | MSR-1(ORF6, *mamF*)[h]<br>MS-1 (ZP_00054712.1)<br>MC-1 (ZP_00288335.1) | 3e – 32<br>3e – 39<br>9e – 22 | amb0412, amb0953, amb0957 | 3e – 49 |
| amb1027 | Bacterial magnetic particle specific iron-binding protein (*mms 5*)[f] | Human herpesvirus 4 (AJ507799-47) | 1e – 16 | MSR-1(ORF7, *mam D*)[h]<br>MS-1 (ZP_00053415.1)<br>MC-1 (ZP_00290176.1) | 1e – 21<br>2e – 23<br>6e – 16 | amb0952 | 2e – 23 |
| amb1028 | Hypothetical protein | *Thermoplasma volcanium* GSS1 (AP000992-291) | 2e – 46 | MS-1 (ZP_00054718.2) | e – 116 | amb1019 | e – 115 |

(*continued on next page*)

Table 2 (*continued*)

| Gene ID | Predicted function[a] | Best hit organisms in non-magnetic bacteria[b] | *E* value[c] | Homologues in magnetic bacteria[b] | *E* value | Paralogues in AMB-1 | *E* value |
|---|---|---|---|---|---|---|---|
| amb1029 | Serine/threonine protein kinase | *Burkholderia pseudomallei* (BX571965-578) | 1e − 40 | MS-1 (ZP_00054716.1) | – | amb1021 | e − 103 |
| amb1030 | FOG:HEAT repeat | *Methanosarcina acetivorans* (AE010948-5) | 3e − 06 | – | – | amb1031 | 9e − 22 |
| amb1031[e] | Hypothetical protein | – | | – | – | amb1030 | 2e − 22 |

[a]Name in parentheses refer to the previously described gene in *Magnetospirillum* sp. AMB-1.
[b]The accession no. of each homologue is shown in the parentheses. In the case of MSR-1, ORF no. and product name in accession no. BX571797 is described. MSR-1, *Magnetospirillum gryphiswaldense*; MR-1, *Magnetospirillum magnetotacticum* strain MS-1; MC-1, *Magnetococcus* sp. strain MC-1; MV-1, magnetite-containing magnetic vibrio strain MV-1.
[c]The *E* value is the value of the top hit in some paralogue.
[d]Gene specific to magnetotactic bacteria.
[e]Gene specific to *Magnetospirillum* sp. AMB-1.
[f]Gene involved in iron metabolism.
[g]Gene involved in ichemotaxis.
[h]amb0951-amb977 correspond to a determined 35-kb cluster from *Magnetospirillum gryphiswaldense*. Schubbe et al. [5]. Each homologue to amb0944 and amb1001 are independently predicted from the drafft sequences of MS-1.

positions nt 997403–998535 and nt 1095895–1097027, respectively (Fig. 2a). The alignment of the two 1.1 kb repetitive sequences shows a few mismatches of nucleotides in the sequences (Supplementary Figure S1). The mismatches are thought to be an accumulation of complementary errors after excision, suggesting that the excision occurred in the region flanking the mismatches.

The region consisted of 98493 bp including 99 ORFs (Fig. 2b, Table 2) and its GC content (61.0%) was lower than that of the total genome (65.1%). Furthermore, orthologs among other magnetic bacteria are concentrated in the region (Table 2). As mentioned above, the deleted region has signature characteristics of a genomic island. A similar prediction has been suggested in other magnetic bacteria [5], but this is the first report to reveal the entire structure and to observe the deletion directly.

### 3.4. Comparative genome analysis with magnetic or non-magnetic bacteria

Magnetic bacteria are distributed over a heterogeneous group of gram-negative bacteria with diverse morphologies and habitats [16–18]. The wide diversity of these organisms suggests that their magnetic properties have no taxonomic significance. Comparative genomic approaches will reveal common factors for magnetosome formation or magnetotaxis. Unfortunately, the genome sequencing of microaerobe *M. magnetotacticum* MS-1 or *Magnetococcus* sp. MC-1 (JGI Microbial Genomics, http://genome.jgi-psf.org/microbial/) has not been completed, but the draft sequences are comparable. Moreover, an 80-kb cluster encoding magnetosome-specific proteins was described in a non-magnetic spontaneous mutant of *M. gryphiswaldense* MSR-1 [5], and a 35-kb sequence of the 80-kb cluster was determined. Thus, 4559 ORFs deduced from the whole genome of *Magnetospirillum* sp. strain AMB-1 were comparatively analyzed with those three magnetic species and other 146 non-magnetic bacteria and 16 archea. The results show that there were 1159 genes specific to *Magnetospirillum* sp. strain AMB-1, not classified in an ortholog group with the 162 non-magnetic bacteria and archea (Group 3 in Fig. 3). Moreover, there were 488 common genes between *M. magnetotacticum* MS-1, *Magnetococcus* sp. MC-1, and *Magnetospirillum* sp. strain AMB-1 (Group 2 in Fig. 3). Consequently, 2912 genes of the total 4559 genes are classified in ortholog group with other bacteria (Group 1). The resulting 488 genes were classified in COG category and 338 of them are not categorized (Table 3). The circular map represents the classification and location of AMB-1 gene. It shows the GC content, IS location, and putative phage-inserted region within the circle (Fig. 3). Group 2 genes are classified genes in the ortholog group with the three magnetic bacteria. In other words, these genes are common only in magnetic bacteria. Group 2 genes occupied 10.7% of 4559 ORFs, but in the 98 kb genomic region, the genes occupied 44.8% of 99 ORFs (Table 2). This was the highest concentration of such genes in the whole genome. The 98 kb region also encodes magnetosome-specific proteins [9], and it may suggest that the 98 kb region was most related to other magnetic bacteria. Therefore, the region could be derived by lateral gene transfer, which introduced genes required for magnetosome formation in a common ancestor.

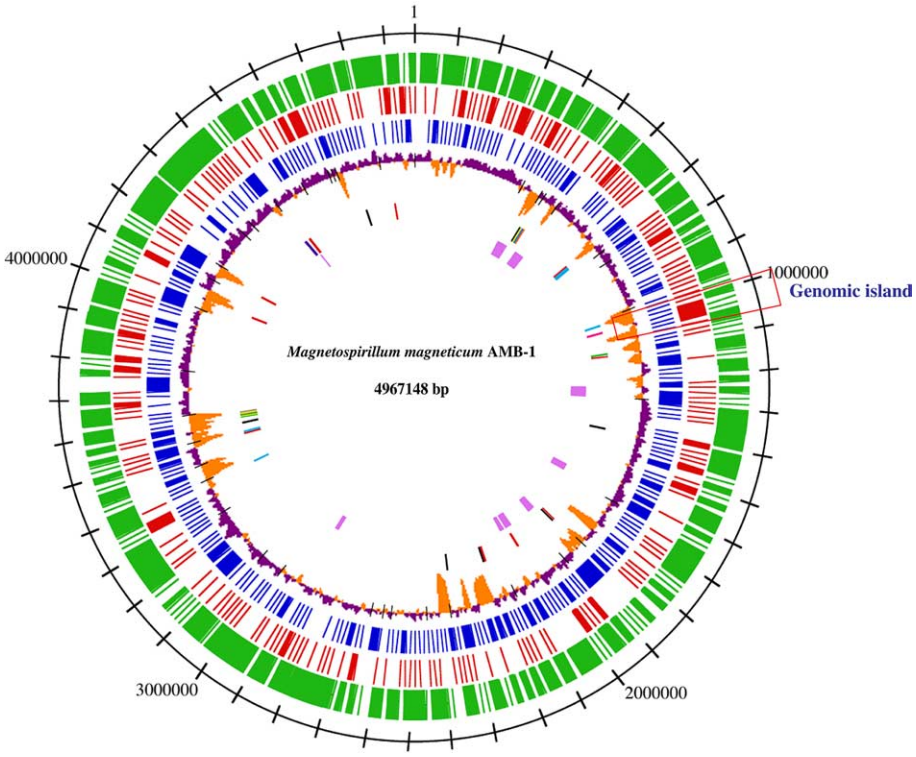


Fig. 3. Circular representation of the gene classification reflected by comparative genome analysis of *Magnetospirillum* sp. strain AMB-1 and other magnetic bacteria and non-magnetic bacteria. The outer circle represents locations of orthologs (Group 1) among AMB-1 and 162 non-magnetic bacteria. The second circle represents locations of orthologs (Group 2) among AMB-1 and other magnetic bacteria and exclude the Group1 genes. The third circle represents AMB-1 specific genes (Group 3), which means the genes excluded Group 1 and Group 2 genes. The fourth circle represents GC content and tRNA location – purple indicates higher than average, orange indicates less than average. The fifth circle represents insertion sequence (IS) elements (These colors were described in [9]). The sixth circle indicates the regions encoding phage capsid proteins.

## 4. Discussion

The 98 kb genomic region was identified as the genomic island that leads to the non-magnetic character of *Magnetospirillum* sp. strain AMB-1 when it is deleted. It seems that the island is essential for magnetosome synthesis considering the concentration of orthologs among magnetic bacteria, in which there exist several genes encoding magnetosome specific proteins. This is similar to the strain *M. gryphiswaldense* MSR-1 [5]. However, other non-magnetic bacteria can not gain the ability of magnetosome synthesis induced only by the island. Whole genome comparative analysis showed many orthologs among magnetic bacteria besides the island. Such orthologs share homology with only magnetic bacteria, not with non-magnetic bacteria, which seems to be related to a function that non-magnetic bacteria do not possess – >magnetosome synthesis. Furthermore, MagA and AOR gene cluster located outside the island have been identified as genes related to magnetosome synthesis [19,20]. Thus it is thought that coordination of the island and other genes is needed to induce magnetosome synthesis.

The genomic island makes a contribution to acquisition or development of environmental adaptation. At the same time it is an invader, meaning that it interrupts the host life system. The island probably also interrupted the AMB-1 life system, and magnetosomes were synthesized. The island null mutant of AMB-1 showed increased growth in comparison with the wild-type. We can conclude from this result that the island may be stressful to the system. But this result is different in *M. gryphiswaldense* MSR-1 [5]. The mutant with a deletion in a similar region showed impaired growth. It might be that the cultivation conditions for AMB-1 and MSR-1 were different from each other, or the differential compatibility of the host and the genomic region. In the latter case, the MSR-1 life system would become dependent on the ''magnetosome island'' for its evolution.

Primers designed in this study could be used for easy detection of the island lost in spontaneous mutants. Using them, one can avoid the mimic generated in the gene knock-down experiments. Additionally, suggestion of island excision by integrase activity would lead to recombinant cells that could not delete the island by integrase inactivation.

The island seems to be derived from lateral gene transfer considering the typical structure of a genomic island and the dynamics after excision from the chromosome. There was no other region where low GC content and Group 2 genes were concentrated except the island. It is concluded that an ancestor of magnetic bacteria contacted another bacterium that possessed the island years ago diverging to AMB-1, MS-1, MC-1 and MSR-1. Further analysis for the origin of magnetosome synthsis will require other organisms possessesing the island or a similar island.

Table 3
COG functional category of common genes among *Magnetospirillum* sp. strain AMB-1 and other magnetic bacteria

| | Common genes in magnetic bacteria |
|---|---|
| *Information storage and processing* | |
| Translation, ribosomal structure and biogenesis | 3 |
| Transcription | 19 |
| DNA replication, recombination and repair | 6 |
| *Cellular processes* | |
| Cell division and chromosome partitioning | 3 |
| Cell envelope biogenesis, outer membrane | 12 |
| Cell motility and secretion | 10 |
| Posttranslational modification, protein turnover, chaperones | 11 |
| Inorganic ion transport and metabolism | 14 |
| Signal transduction mechanisms | 65 |
| *Metabolism* | |
| Energy production and conversion | 7 |
| Amino acid transport and metabolism | 3 |
| Nucleotide transport and metabolism | 0 |
| Carbohydrate transport and metabolism | 2 |
| Coenzyme metabolism | 7 |
| Lipid metabolism | 7 |
| Secondary metabolites biosynthesis, transport and catabolism | 5 |
| *Poorly characterized* | |
| General function prediction only | 20 |
| Function unknown | 12 |
| Others | 338 |
| Total | 488 |

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2006.01.003.

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